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INVESTIGATION OF THE EFFICACY OF SEVERAL COMMON HERBICIDES ON SUGAR BEET SEEDLING DAMPING-OFF DISEASE IN GREENHOUSE*

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ABSTRACT

Seedling damping- off is one the most important disease of sugar beet. In this study, the effect of five commonly herbicides were investigated on pathogens of sugar beet seedling damping- off in greenhouse. *Rhizoctonia solani* (R.S.K.4 and R.S.K.5) and *Fusarium proliferatum* (F.P.K.1) are used as pathogens which its isolation have been approved during research projects in the growing season among 2007 to 2009. Greenhouse study was done in factorial experiment in a randomized complete block design. Factors included sugar beet cultivars in four levels (Shirin, Jolgah, Gaduk and 31145) and herbicides (no herbicides in healthy control, no herbicides in unhealthy control, Betanal AM, Betanal Progress AM, Betanal Progress OF, Safary and Pyramin). The effect of experiment treatments on controlling damping- off disease was evaluated by determining percent of healthy seedling during 15, 30, 45 and 60 days after planting. Greenhouse results about the experiment of the effects of cultivar and herbicide factors separately on percent of healthy seedling showed that there was not significant difference between various treatments. However, the results related to the experiment of the interaction effects between two above-mentioned factors on percent of healthy seedling showed that there was significant difference between different treatments in the 5% level. In this experiment, the maximum percent of healthy seedling belonged to treatments related to Shirin, Jolgah and 31145 when Betanal AM, Betanal Progress AM and Betanal Progress OF had been applied in them.

KEYWORDS: Herbicides, Seedling Damping-Off Disease, Sugar Beet

INTRODUCTION

Beta vulgaris is one of the most important agricultural crops which is cultured as a main agriculture in most of countries in the world especially Iran. Importance of this agricultural crop is due to different application in human and animals. In Iran, there is 200.000 hectare infield to culture *Beta vulgaris*, but unfortunately performance of this product compared to other countries is low. One of most important reason in low performance of this product is different disease (Sheikholeslami *et al*, 2005).

Sugar beet seedling damping- off is one of most prevalent disease for this product in Iran (Momeni *et al*, 2002; Babai et al, 2004) and outside the country (Luterbacher *et al*, 2005). Environmental factors play important role to develop and incidence of damping- off pathogen factors. In addition, humidity, heat, soil texture and soil pH, chemical

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combinations like herbicides are most important factors influence on damping- off disease (Heydari *et al*, 2008). Based on conducted studies outside the country, some herbicides like Prometryn, Gezaguard brand name increase damping- off disease in the cotton (Heydari and Misaghi, 1998), while other kinds like Pendimethalin by Stomep brand name decrease damping- off disease in the soybean (Harikrishnan and Yang, 2001).

So far, a lot of reports were mentioned in term of plant disease and herbicides and interactions which lead to increase or reduce disease are related to pathogen– plant- herbicides systems. Based on studies in USA, there is correlation between increasing used herbicides in the year and increasing *Beta vulgaris* decay. Some researchers have reported herbicides usages increased fungus of *Rhizoctonia solani*, damping- off and root decay in *Beta vulgaris* significantly (Ruppel *et al*, 1982)

In other study, effect of PCA herbicides (Polycyclic Alkanoic Acid) was examined on damping- off disease of *Beta vulgaris*. Results of this study showed, these herbicides increase damping- off disease if applied in the champ with sandy soil texture or clay and artificial contamination *R.solani* and *Sclerotinia Rolfsii*. In other hand, in champs which have been contaminated by *Fusarium solani* manually, these herbicides increased disease, while clay texture decreased disease significantly (Maareg *et al*, 1989). Bradley *et al*. (2002) showed herbicides like Pendimethalin, Acifluorfen and Imazethapyr increased raizoctonia decay in soy root. Effect of Glyphosate herbicides was examined on barley root decay and showed this herbicide increase disease (Babiker *et al*, 2011).

In same study in American Arizona, Heydari et al, 2008 examined effects of some herbicides on fungicide efficiency to decrease damping- off in cotton. Results of the study showed Pendimetalin herbicide increase efficiency of fungicides like metalaksil, terydimnol and tiram to reduce *R. solani* pathogens on cotton seedling, while simultaneous application of Metalin and Prometrin reduce fungicide ability to decrease damping- off disease. Also, results of above study showed Trifloralin (Terfelan) herbicide have not effected on mentioned fungicides (Heydari *et al*, 2008).

Also, in Iran studies was conducted on simultaneous application of herbicides and antagonist fungus on damping- off disease on cotton fields in Moghan and Neishabour (Naraghi *et al*, 2012). Results of this study showed simultaneous application of Terfelan and Etal floralin (Sonalan) herbicides with affected treat of antagonist fungus (seed embedding and increase to soil) decrease damping- off disease in the cotton. Also, laboratory experiments was done on five prevalent herbicides in *Beta virgulas* (2,4,D, Desmedifam, Diclophop- Methyl, Cholodinphop and Teralkoksidum on some fungi species such as *Ceratocystis radicicola*, *Fusarium culmorum*, *Fusarium graminearum*, *F. proliferatum*, *Fusarium oxysporum*, *Gaeumannomyces graminis*, *Macrophomina phaseolina*, *Phytophthora* sp, *Pythium* sp, *R. solani*, *Sclerotinia sclerotiorum*, *Talaromyces flavus*, *Trichoderma koningii*, *T. longibrachiatum*, *T. hamatum*, *T. harzianum*, *Trichoderma* sp. and *Verticillium dahlia* (Pakdaman *et al*, 2002). Results of recent study showed, 2, 4, D, Desmedifam and Diklophop-Methyl had most effect on fungi antagonist and all of mentioned herbicides could prevent growing fungus related to Omist categorization (*Pythium and Phytophthora*). In this study, fungi antagonist effects of two herbicides like Diklophop- Methyl and Chlodinaphop were reported first time.

The objective of this study was to select herbicides with more ability to decrease seedling damping-off for their application in the sugar beet fields in future.

MATERIALS AND METHODS

Greenhouse Examination

Soil Preparing

In this study, sugar beet fields soil located in Meshkin Abad area in Alborz Province of Iran was used. Although this place was contaminated to damping off disease, but for assure disease and symptom, soil inoculation was conducted manually based on below processes:

- **Selecting Pathogenic Agents**

In this study, *R. solani* (R.S.K.5 and R.S.K.4) and *F. proliferatum* (F.P.K.1) which were examined in previous study (Naraghi *et al*, 2014) were selected as pathogenic agents of seedling damping-off disease.

- **Soil Inoculation with Pathogenic Agents**

Soil inoculation with *R. solani* was done based on the method described by Khalil *et al*. (2003). At first, one 500 ml flask consists of 50 gr barely seed and 40 ml water were placed in autoclave for 30 minutes. Then two or three pieces of each fungus was transferred to flask and mixed completely. Flasks were placed in incubator with 30^0C for three weeks to grow and develop scrod. When coverage of barely seed of mycelium of fungus was observed, containers in each flask were speared to dry in laboratory temperate and were used as *R. solani* pathogen. Embedding was done as one kilogram of soil and one gram of pathogenic inoculum.

Soil inoculation with *F. proliferatum* was done based on the method described by Mikhail *et al*. (2009). At first, one 500 ml flask consists of 100 gr corn seed and 80 ml water were placed in autoclave for 30 minutes. Then two or three pieces of each fungus was transferred to flask and mixed completely. Flasks were placed in incubator with 300C for three weeks to grow and develop scrod. When coverage of corn seed of mycelium of fungus was observed, containers in each flask were speared to dry in laboratory temperature and were used as *F. proliferatum* pathogen. Embedding was done as one kilogram of soil and 50 gram of pathogenic inoculum.

The Effect of Sugar Beet Cultivar and Herbicide Factors Separately and Interaction Effects between Factors on Healthy Seedling Percent in the Greenhouse Conditions

In this study, four herbicides were used to overcome broadleaf weed and four herbicides were used to overcome narrow leaf weed. Formulation, commercial name, consumption amonth and application time of used herbicides were given in Table 1:

Table 1: Commercial Name, Formulation, Consumption a Month and Application Time of Used Herbicides

Commercial Name of Herbicide	Formulation	Consumption a Month	Application Time	Used Resource
Betanal AM	EC= 4.27%	4.5 l /hec	2 to 4 leaves of *Beta vulgaris*	Kudsk and Mathiassen, 1994
Betanal Progress AM (Fenmedifam 60 gr/l+ Desmedifam 60 gr/l + Autofomozit 60 gr/l)	EC= 4.27%	4 l /hec	2 to 4 leaves of *Beta vulgaris*	Kucharski, 2009
Betanal Progress OF (Fenmedifam 91 gr/l+ Desmedifam 71 gr/l + Autofomozit 112 gr/l)	EC= 4.27%	3–3.5 l/hec	2 to 4 leaves of *Beta vulgaris*	Kucharski *et al*, 2008

Table 1: Cond.,

Safary (Trisulfuron methyl)	DF= 50%	30 gr Safari + 200 ml Moyan + 2 l Betanal AM for one hectare	Cotyledon of *Beta vulgaris*, repetition after one week	Mechrafi *et al*, 2001
Pyramin (Chloridazon)	DF= 65%	4- 5 kg in 400 l of water for one hectare	pre-growth and 2 to 4 leaves of *Beta vulgaris*	Moliszewska, 2000

Greenhouse examination was done in factorial experiment in a randomized complete block design. Factors included *Beta vulgaris* cultivars in four treatments (Shirin, Jolgah, Gaduk and 31145) and different common herbicides in seven treatments (no herbicide in healthy control, no herbicide in unhealthy control, Betanal AM, Betanal progress AM, Betanal Progress OF, Safari and Pyramin). In this experiment, four repetitions were mentioned and each repetition included a pot in 20 centimeters diameter consisting of ten seeds of the used cultivar in the experiment in term of treatment. For healthy control, autoclaved soil of sugar beet field in Karaj and for other treatments, Karaj field soil with artificial inoculation were used. All herbicides were applied as spray and needed amonth for 400 liter of water related to one hectare (Seefeldt *et al*, 2001).

Based on given all mentioned amonth in Table 1 for herbicides in one hectare equivalent to 10^4 square meters, needed amonth for pot with 3×10^{-2} square meters were calculated. For example, for herbicide application with consumption amonth 2 l/hec in a pot with 20 centimeters diameter, six microliter of herbicide were mixed to 1200 microliter of water and spraying was done in consumption time. All pots were placed in greenhouse in 20^0C temperature. Evaluation of disease occurrence in different treatments have been carried out in 15, 30, 45 and 60 days after planting as percent of healthy seeding. According to the results of above-mentioned experiment, three cultivars and three herbicides were selected for future field studies to reduce sugar beet seedling damping off disease.

Separating Pathogenic Fungi of Unhealthy Plants

In this section, crown and root of unhealthy seedling were sterilized by bleaching solution 10% (consisting of hypochlorite sodium 5%) for 10 to 60 seconds after washing texture in the water for several minutes. After washing three times in a sterilized Petri dish with sterilized water and dry it with sterilized paper, texture were segmented and cut pieces were removed of healthy texture. Then, these pieces were transferred to Petri dishes consisting of Water- Agar (W.A.) medium. Petri dishes were placed in incubator in 22 to 25^0C. After 12 to 48 hours and observing fungal colonies, macroscopic and microcopic features explained by Gonzales Garcia *et al*. (2008) was used to identify *R. solani* and macroscopic and microcopic features explained by Nelson *et al*. (1983) and Sutton *et al*. (1998) were used to identify *F. proliferatum*

Pathogenicity Test

This test was done based on Koch principles. After separating pathogenic fungi of damping off seedling, 75 hour cultures of these factors was added to autoclaved soil of pots consisting of ten seeds of sugar beet sensitive cultivar (Shirin). The experiment evaluation carried out from one week to one month after planting based on healthy seedling percent.

RESULTS

The Effect of Sugar Beet Cultivar and Herbicide Factors Separately and Interaction Effects between Factors on Healthy Seedling Percent in the Greenhouse Conditions

Cultivar Factor Effect

Results of the effect of cultivar factor in 15, 30, 45 and 60 days after planting showed that this experiment was significant in 1% probable level. Results of grouping means of healthy seedling percent showed that in all times, highest and lowest mean belonged to Jolgah and Gaduk cultivars respectively (Table 2).

Table 2: Grouping of Means of Sugar Beet Healthy Seedling Percent in Experiment Related to Cultivar Factor during 15, 30, 45 and 60 Days after Planting in Greenhouse Conditions

Cultivar Factor	Percent of Healthy Seedling *			
	15 Days after Planting	30 Days after Planting	45 Days after Planting	60 Days after Planting
31145	41.78b	33.21a	38.57b	38.75 b
Jolgah	56.42a	37.50a	56.07a	54.28 a
Shirin	34.64c	22.14b	37.50b	37.58b
Gaduk	33.92c	26.07b	29.28c	32.14 b

* There is no significant different in 1% probable level between data of each column with similar letters.

Herbicide Factor Effect

Results of the effect of herbicide factor in 15, 30, 45 and 60 days after planting showed that this experiment was significant in 1% probable level. Results of grouping means of healthy seedling percent showed that all times, highest mean have been belonged to healthy control and affected treatments of Betanal AM, Betanal progress AM and Betanal Progress OF (Table 3).

Table 3: Grouping of Means of Sugar Beet Healthy Seedling Percent in Experiment Related to Herbicide Factor during 15, 30, 45 And 60 Days after Planting in Greenhouse Conditions

Herbicide factor	Percent of Healthy Seedling *			
	15 Days after Planting	30 Days after Planting	45 Days after Planting	60 Days after Planting
Betanal AM	40.62 c	29.37b	43.75b	43.12b
Pyramin	36.85 c	18.75c	30.00c	31.87c
Safary	24.37c	15.00c	16.87d	55.50d
Betanal Progress AM	38.75 c	24.37b	38.75bc	42.50b
Betanal Progress OF	50.00b	27.50b	48.12b	49.37b
Unhealthy control	40.00 c	27.50b	36.87c	39.37bc
Healthy control	61.25a	65.00b	65.00b	61.25a

* There is no significant different in 1% probable level between data of each column with similar letters.

Interaction Effects between Cultivar and Herbicide Factors

Results of the interaction effects between cultivar and herbicide factors during 15, 30, 45 and 60 days after planting showed that the experiment was significant in 5% probable level. Results of treatments grouping showed that highest percent of health seeding belonged to 31145, Jolgah and Shirin cultivars which had been applied Betanal AM, Betanal progress AM and Betanal Progress OF herbicides (Table 4).

Table 4: Grouping of Means of Sugar Beet Healthy Seedling Percent in Experiment Related to Cultivar and Herbicide Factors during 15, 30, 45 and 60 Days after Planting in Greenhouse Conditions

Cultivar and Herbicide Factors	Percent of Healthy Seedling *			
	15 Days after Planting	30 Days after Planting	45 Days after Planting	60 Days after Planting
31145- Betanal AM	60.00ab	50.00b	60.00ab	60.00ab
31145- Pyramin	30.00bc	12.50d	27.50c	32.50bc
31145- Safary	30.00bc	12.50d	12.50d	10.00d
31145- Betanal Progress AM	50.00b	35.00bc	47.50d	47.50b
31145- Betanal Progress OF	47.50b	35.00bc	52.50b	55.00b
31145- Unhealthy control	25.00c	30.00b	20.00cd	32.50bc
31145- Healthy control	50.00b	50.00b	50.00b	50.00b
Jolgah- Betanal AM	57.50b	45.00b	67.50ab	65.00bc
Jolgah- Pyramin	62.50ab	12.50d	40.00b	40.00b
Jolgah- Safary	35.00bc	20.00cd	35.00bc	35.00bc
Jolgah- Betanal Progress AM	40.00b	20.00cd	40.00b	40.00b
Jolgah- Betanal Progress OF	67.50ab	40.00b	65.00ab	60.00bc
Jolgah- Unhealthy control	52.50b	32.50bc	52.50b	52.50b
Jolgah- Healthy control	92.50a	92.50a	92.50a	92.50a
Shirin- Betanal AM	32.50bc	10.00d	32.50bc	32.50bc
Shirin- Pyramin	37.50bc	25.00c	37.50bc	40.00b
Shirin- Safary	12.50d	10.00d	17.50d	25.00c
Shirin- Betanal Progress AM	40.00b	25.00c	45.00b	42.50b
Shirin- Betanal Progress OF	47.50b	20/00cd	40.00b	42.50b
Shirin- Unhealthy control	27.50c	10.00d	35.00bc	32.50bc
Shirin- Healthy control	45.00b	55.00b	55.00b	50.00b
Gaduk- Betanal AM	12.50d	12.50d	15.00d	15.00d
Gaduk- Pyramin	30.00bc	25.00c	15.00d	15.00d
Gaduk- Safary	20.00cd	10.00d	15.00d	20.00cd
Gaduk- Betanal Progress AM	25.00c	17.50d	22.50cd	32.50bc
Gaduk- Betanal Progress OF	37.50bc	17.50d	35.00bc	40.00b
Gaduk- Unhealthy control	55.00b	37.50bc	40.00b	40.00b
Gaduk- Healthy control	57.50b	62.50b	62.50b	62.50b

* There is no significant different in 5% probable level between data of each column with similar letters.

Microscopic and Macroscopic Studies of *R. Solani* and *F. Proliferatum* Isolated from Unhealthy Plants

Microscopic study of *R.solani* showed that this fungus had brown hyphae. There were vertical branches closed to some hyphae cells which are compressed in the production point (Figure 1). Also, dark brown cell with thick cells named Monolioed was produced which were placed as a chain (Figure 2). In the old phase, sclerotia had been produced with

integrated texture and different sizes and forms by aggregating Moniolioeids cells (Figure 3). In Macroscopic study, colonies color was brown in PDA (Potato Dextrose Agar) medium (Figure 4).

Figure 1: Vertical Branches in *Rhizoctonia solani*

Figure 2: Formation of Monlioeid in *Rhizoctonia solani*

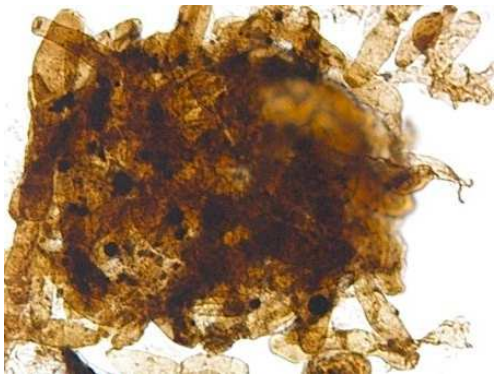

Figure 3: Aggregation of Monilioeids and Formation of Sclorotia in *Rhizoctonia solani*

Figure 4: *Rhizoctonia Solani* Colony with Brown Color on PDA Medium

In microscopic study of *F. Proliferatum*, this fungus had clear hyphae. Conidiophores were shown with middle length as simple or branch with monofiald or polyfiyalid cells. There were Microconidia as abundant with 1.3-2.5 × 4.5-10.5µ in diameter on monophialids or polyphialids (Figure 5). In this fungus, macroconidium and clamydospore were not observed. In macroscopic study, the color of the fungus colonies on PDA medium during dark and light period was white first and gradually converted to purple (figure 6). Spordochium has not been formed on PDA medium.

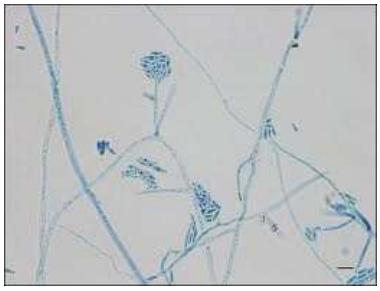

Figure 5: Microconidi Aggregation of *Fusarium proliferatum* at the End of Conidiophores

Figure 6: *Fusarium proliferatum* Colony on PDA Medium: it was White and Gradually Converted to Purple

Pathogenicity Test

In this section, mean of healthy seedling percent was 40% in treatment affected by pathogenic agents. This value was 100% in the control.

DISCUSSIONS

Overall results of this study indicate that it may be possible to promote health and growth of sugar beet using suitable cultivars and herbicides.

The greenhouse results of the study in theeffect of the cultivars on damping off disease showed that some cultivars such as Jolgah and Gaduk were resistant to the disease and number of healthy seedling in the treatments affected by above- mentioned cultivars were higher significantly. In this subject, some studies showed that damping off disease induced by *Aphanomyces cochiloides* has been decreased significantly (Bruntner and Windels, 2003).

In other hand, the effect of the herbicides on number of healthy seedling showed that some herbicides such as Safary and Pyramin increased damping off disease. Results of previous study showed that this was emerged by the effect of the herbicides on plant resistance reduce (Neubauer and Avizohor- Hersnenson, 1973), increase pathogenic activities (EL-Khadem and Papavizas, 1984) or decreasing useful microorganism numbers in the soil (Altman and Cambell, 1977).

Also, the results related to interaction effects between cultivar and herbicides factors showed that highest percent of healthy seedling was belonged to 31145, Jolgah and Shirin which Betanal AM, Betanal progress AM and Betanal Progress OF had been applied. In similar study about the effects of several herbicides on cotton seedling damoing-off disease caused by *R. solani*, was shown that Avanegard cultivar with Alaker and Ethalfluralin herbicides didn't affect damping off disease, while Sepid cultivar with Ethalfluralin herbicide and Varamin cultivar with Alakler herbicide decreased and increased disease incidence respectively (Modiri *et al*, 2013).

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